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# Tissue integration of zirconia and titanium implants with and without buccal dehiscence defects-A histologic and radiographic preclinical study

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Abstract: OBJECTIVE To histologically and radiographically evaluate soft (primary outcome) and hard tissue integration of two-piece titanium and zirconia dental implants with/without buccal dehiscence defects. MATERIALS METHODS In six dogs, five implants were randomly placed on both sides of the mandible: (a) Z1: a zirconia implant (modified surface) within the bony housing, (b) Z2: a zirconia implant (standard surface) within the bony housing, (c) T: a titanium implant within the bony housing, (d) $Z1_D$ : aZ1implantplacedwithabuccalbonedehiscence(3mminheight, identicalwidthtoimplantbody), and(e)T_D : atitaniumimplantplacedwithabuccalbonedehiscence.Twoweeksofhealingand6monthsofloadingwereappliedoneachhemi mandible, respectively.RESULTSThemedianlevelofthemargomucosaeshiftedmoreapicallyovertimeinallgroups(borde −0.52mmandT_D : −1.26mm).Themedianheightoftheperi−implantmucosaingroups$Z1_D$andT_Dwasgreatestat2week implantmucosawasstatisticallysignificantonlyforgroupT_Dovertime(−1.45mm).Z1demonstratedahigherbone− to−implantcontactcomparedtoZ2andT.Minimalchangeofradiographicmarginalbonelevelsinallgroupswasobserved(< 1mm).CONCLUSIONWhenbuccaldehiscencewaspresented, titaniumimplantspresentedsignificantlossofperi− implantmucosalheightcomparedtozirconiaimplantswithamodifiedsurface, duetogreaterapicalshiftofthemargomucos

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# Tissue integration of zirconia and titanium implants with and without buccal dehiscence defects – a histologic and radiographic preclinical study

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**Running title:** Tissue integration of zirconia and titanium implants

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**Author Contributions:**

Daniel S. Thoma, Ui-Won Jungand Ronald E. Jung conceived the idea; Daniel S. Thoma, Hyun-Chang Lim, Kyeong-Won Paeng and Ui-Won Jungperformed the experiment; Kyeong-Won Paeng and Ui-Won Jungcollected the data; Daniel S. Thoma, Hyun-Chang Lim and Ronald E. Junganalyzed the data; Hyun-Chang Lim wrote the original draft of the manuscript; Daniel S. Thoma, Christoph H.F. Hämmerle and Ronald E. Jung reviewed and edited the manuscript; Christoph H.F. Hämmerle supervised the study.

## Abstract

**Objective:** To histologically and radiographically evaluate soft (primary outcome) and hard tissue integration of two-piece titanium and zirconia dental implants with/without buccal dehiscence defects.

**Materials & Methods:** In 6 dogs, five implants were randomly placed on both sides of the mandible: i) Z1: a zirconia implant (modified surface) within the bony housing, ii) Z2: a zirconia implant (standard surface) within the bony housing, iii) T: a titanium implant within the bony housing, iv) Z1_D: a Z1 implant placed with a buccal bone dehiscence (3mm in height, identical width to implant body), and v) T_D: a titanium implant placed with a buccal bone dehiscence. Two weeks of healing and 6 months of loading were applied on each hemi-mandible, respectively.

**Results:** The median level of the margo mucosae shifted more apically over time in all groups (borderline statistical significance in groups Z1_D: -0.52 mm and T_D: -1.26 mm). The median height of the peri-implant mucosa in groups Z1_D and T_D was greatest at 2 weeks and 6 months, but the linear change in the peri-implant mucosa was significant only for group T_D over time (-1.45 mm). Z1 demonstrated a higher bone-to-implant contact compared to Z2 and T. Minimal change of radiographic marginal bone levels in all groups were observed (less than 1 mm).

**Conclusion:** When buccal dehiscence was presented, titanium implants presented significant loss of peri-implant mucosal height compared to zirconia implants with a modified surface, due to greater apical shift of the margo mucosae. A modified zirconia surface enhanced osseointegration.

**Keywords:** dehiscence, osseointegration, peri-implant mucosa, titanium, zirconia

## Introduction

Titanium and titanium-based alloys are considered as the gold standard materials for dental implants (Hisbergues, Vendeville, Vendeville, 2009), due to excellent tissue response and mechanical properties (Steflik, Corpe, Young, Buttle, 1998). A large number of clinical studies demonstrated high implant survival rates for various clinical indications in partially edentulous patients (Jung, Zembic, Pjetursson, Zwahlen, Thoma, 2012; Pjetursson, Thoma, Jung, Zwahlen, Zembic, 2012; Roccuzzo, Bonino, Gaudioso, Zwahlen, Meijer, 2012). Reported disadvantages and limitations for titanium implants, however, included hypersensitivity, corrosion, esthetic problems and susceptibility to bacterial peri-implant adhesion (Cionca, Hashim, Mombelli, 2017).

Zirconia was introduced as an alternative material to compensate for some of these limitations and to meet patient demands for metal-free reconstructions. Biological properties of zirconia implants were thoroughly investigated on both the hard and soft tissue levels, and also in comparison to titanium implants. Scientific evidence demonstrated no significant differences in the degree of osseointegration with or without bone augmentation procedures between zirconia and titanium implants (Benic, et al., 2017; Hafezeqoran, Koodaryan, 2017), thereby indicating the suitability of zirconia implants for clinical use. Moreover, even though limited, experimental data demonstrated dimensional and morphologic similarities in the peri-implant mucosa between zirconia and titanium implants (Tete, Mastrangelo, Bianchi, Zizzari, Scarano, 2009; Thoma, et al., 2015; Welander, Abrahamsson, Berglundh, 2008).

Observational studies indicated that dental implants may not always be completely embedded by bone even though demonstrating clinically successful tissue integration at 7 and 10 years respectively (Benic, et al., 2012; Kuchler, Chappuis, Gruber, Lang, Salvi, 2016). This observation may be due to continuous remodeling processes of the alveolar ridge or failing GBR procedures, but also suggests that the peri-implant soft tissues can compensate for missing hard tissue. In a recent clinical trial, buccal bony dehiscence defects around titanium implants were intentionally left without guided bone regeneration, thereby exposing the rough titanium surface to the peri-implant soft tissues (Jung, et al., 2017). Healthy and stable soft tissues, but more marginal bone loss at sites with bony dehiscence compared to augmented sites were reported at 18 months of follow-up. Due to an excellent biocompatibility, favorable soft tissue integration and a minimized plaque accumulation (Tete, et al., 2009; Welander, et al., 2008), speculation has been raised that zirconia implants might be more favorable than titanium implants in case of (un)intentionally exposed implant threads.

The aims of the present study were, therefore, to histologically and radiographically evaluate soft and hard tissue integration of two-piece titanium and zirconia dental implants with and without buccal dehiscence defects.

## Materials and methods

The present study was designed following the ARRIVE guidelines for preclinical research (Kilkenny, et al., 2010). A total of six mongrel dogs (> 2 years old) weighing a maximum of 30 kg were selected. All surgeries were carried out at Yonsei Medical Research Center, Seoul, South Korea. The experimental protocol was approved by the Institutional Animal Care and Use Committee (Approval no. 2017-0333). All dogs were housed at a room temperature of 15–20°C and a humidity of >30% and provided individual access to water ad libitum and a soft diet throughout the entire study period. All animals were checked medically and dentally 2 weeks before the initiation of the first surgery. Supragingival calculus removal and plaque control were carried out prior to the surgical phase. Throughout the experimental period, a soft diet was provided.

### Surgical procedures

A detailed schedule of the interventions is presented in Figure 1.

On the day of the surgery, the general health of all animals was checked and documented. All surgical procedures were performed under general anesthesia. General anesthesia was induced by an intravenous injection of atropine (0.04 mg/kg; Kwangmyung Pharmaceutical, Seoul, Korea) and an intramuscular injection of a combination of xylazine (Rompun, Bayer Korea, Seoul, Korea) and ketamine (Ketara, Yuhan Corporation, Seoul, Korea), then followed by an inhalation anesthesia (Gerolan, Choongwae Pharmaceutical, Seoul, Korea). The postsurgical management included daily irrigation with 0.2% chlorhexidine solution (Hexamedin, Bukwang Pharmaceutical, Seoul, Korea), and an intramuscular injection of antibiotics (20 mg/kg; cefazolin sodium, Yuhan, Seoul, South Korea) and analgesic (Meloxicam, Boehringer Ingelheim, Bogota D.C., Colombia).

The surgical protocol and the validity of the present model were previously described (Lim, et al., 2018).

*Tooth extraction and creation of chronic defects*

All mandibular premolars (P1, P2, P3 and P4) and the first molar (M1) on both sides of the mandible were extracted. For that purpose, a full thickness flap was elevated, and teeth separated to prevent fractures. Following extraction, a one-wall defect was prepared by removing the buccal bone plate in the surgical sites randomized to undergo implant placement with dehiscence defects. In all other sites, the extraction sockets were left intact. Primary wound closure was then obtained.

*Implant placement*

Implant placement was performed in a staged manner on the left and right sides of the mandible to allow for two healing time-points (6 months and 2 weeks, respectively). On one side of the mandible in each dog, a mid-crestal incision made from the canine to the second molar, and mucoperiosteal flaps reflected. Five osteotomies were prepared, and dental implants placed according to the manufacturer's recommendation, thereby allocating 5 experimental groups bilaterally in each dog (n=6 for each healing time-point) and based on a computer-generated randomization list: (Fig. 2a):

(i) Z1: zirconia implant (AXIS HEXALOBE™ with a modified surface, Ø4.0 x 8 mm; AXIS Biodental, Les Bois, Switzerland) placed within the bony housing,

(ii) Z2: zirconia implant (AXIS HEXALOBE™, Ø4.0 x 8 mm; AXIS Biodental) placed within the bony housing,

(iii) T: titanium implant (CAMLOG® SCREW-LINE promote® plus, Ø3.8 x 9 mm, Camlog Biotechnologies, Basel, Switzerland) placed within the bony housing,

(iv) Z1_D: Z1 implant placed with buccal bone dehiscence,

(v) T_D: Titanium implant placed with buccal bone dehiscence.

Z2 surface is a moderately rough surface with Ra ≈ 1.6 µm, achieved by a ceramic injection molding process. Z1 surface is a modified surface from Z2 surface using a hydroxyapatite (HA) coating with biomimetic dimensions.

At least 2 mm of buccal bone was present after implant placement in groups Z1, Z2, and T. In groups Z1_D and T_D, the height of buccal bone dehiscence was 3 mm and the width was identical to the implant body.

For zirconia implants, the final position of the implant shoulder was 0.7 mm above the bone crest. For titanium implants, the border between the rough and smooth surface was located at the level of the alveolar crest (implant shoulder was located 0.4 mm above the bone crest). Healing abutments were then positioned for transmucosal healing and flaps adapted.

*Crown insertion*

After a healing period of 8 weeks following implant placement (Fig. 2b), prefabricated CAD/CAM crowns were inserted on all implants on one side of the mandible (Fig. 2c). In order to fabricate the crowns, one zirconia and one titanium implant were inserted in a plaster model. Then, a PEKK (poly ether ketone ketone) and a hybrid abutment (titanium basis) were connected to the zirconia and the titanium implants. These situations were scanned using an intraoral (3Shape, Copenhagen, Denmark). The scanned images were transferred to a CAD/CAM system (3Shape dental designer, 3Shape) in order to produce crowns with a screw access hole. The crowns were cemented to the abutments extra-orally, and any cement remnant completely removed. The crown-abutment was connected to the implant using a screw.

*Implant placement on opposite side of the mandible*

Thirty weeks after implant placement on one side of the mandible and 2 weeks prior to sacrifice (38 weeks after tooth extraction), dental implants were placed according to the above-mentioned protocol and left for transmucosal healing on the other side of the mandible. Thereby, a healing period of 2 weeks was established. No crowns were inserted on these implants.

*Sacrifice*

After 2 weeks following the second implant surgery, the dogs were sacrificed with an intravenous overdose of sodium pentobarbital, resulting in 6 months of loading on one side of the mandible and 2 weeks of healing after implant placement on the other side of the mandible. Implants and surrounding tissues were macroscopically inspected, and tissue sections containing the implants resected (Fig. 2d).

**Histological preparation**

The harvested tissue sections were immersed in 10% neutral-buffered formalin for 10 days. Then, the specimens were trimmed, dehydrated in ethanol and embedded in methyl methacrylate. A central bucco-oral section for each implant site was prepared at 40-50 µm thickness using a microcutting and grinding technique adapted by Donath and Breuner (Donath, Breuner, 1982). Thereafter, the sections were stained with van Gieson.

**Intraoral radiographs**

Intraoral x-rays were taken using a standardized parallel technique at implant placement, at crown insertion and at sacrifice.

**Analyses**

*Histomorphometric analysis*

The images from the histological slides were observed using a light microscope (Leica DM6000 B; Leica Mikrosysteme, Wetzlar, Germany) equipped with a digital camera (Leica DFC 450; Leica Mikrosysteme), and each image was then digitally scanned for image analysis. The acquired images were processed (Photoshop CS6, Adobe, California, USA) and analyzed (Leica Application Suite Ver. 4.3, Leica Mikrosysteme).

*Peri-implant soft tissue dimensions and bone-to-implant contact*

The following landmarks were identified (Thoma, et al., 2015): (i) the margo mucosa, MM; (ii) the implant shoulder, IS; (iii) the apical extension of the junctional epithelium, aJE; (iv) the first bone-to-implant contact, fBIC; and (v) the bone crest, BC. Using these landmarks, the following linear parameters were measured: (i) the level of the margo mucosa relative to the implant shoulder, MM-IS; (ii) the length of the junctional epithelium, MM-aJE; (iii) the length of peri-implant mucosa, MM-fBIC; (iv) the distance between IS and fBIC, subtracted by 0.7mm (zirconia implant) or 0.4mm (titanium implant), due to the two different insertion protocols and height of the implant collars, IS-fBIC_c; and (v) the distance between the bone crest and the fBIC, BC-fBIC (Fig. 3a).

A region of interest (ROI) was defined with a length of 4mm on the buccal and lingual side of each implant. Then, the mean bone-to-implant contact (BIC, %) was calculated on the buccal and lingual side and then averaged (Fig. 3b).

*Radiographic analysis*

All digital x-ray images were transferred into a software program (Photoshop CS6, Adobe, California, USA), and the first bone-to-implant contact (fBIC) was measured on the mesial and distal surfaces using the known pitch distance between the implant threads. Marginal bone level changes were calculated between the three time-points (implant placement, crown insertion, sacrifice).

## Statistical analysis

The statistical analyses were performed with the software SAS 9.4 (SAS Cary N.C. USA)

The metric variables with mean, standard deviations, median and quartiles were described. Since several treatments and measurements are clustered within the same dog, mixed linear models were applied for the analyses of the fixed effects as time and treatment, including their interaction. However, this interaction was not significant, also possibly due to the small sample size. Hence, only the time or the treatment effect was investigated for the corresponding subgroups of data.

Pairwise comparisons in case of a significant effect are adjusted for the multiple testing by Bonferroni correction. If no significant differences were observed, not-adjusted p-values were presented.

# Results

### Clinical observation

All dogs remained healthy during the entire study period and the healing was uneventful in all experimental sites. No implants or crowns were lost.

### Histomorphometric analyses

The data of the linear measurement are presented in Table 1. The data for the percentages of the bone-to-implant contact (BIC) are presented in Table 2.

Representative histologic views at 2 weeks of healing and at 6 months of loading are presented in Figure 4.

#### ***Peri-implant soft tissue dimensions***

The median level of the margo mucosae was located coronally relative to the implant shoulder (MM-IS) on the buccal side, ranging between 1.63 mm (Q1= 1.57; Q3= 3.09) for group Z1_D and 2.64 mm (1.68; 3.47) for group T in the 2-week healing groups, and between 1.23 mm (1.01; 1.58) for group Z1_D and 1.63 mm (1.13; 2.11) for group T in the 6-month loading groups (intergroup comparisons $p>0.05$). Over time, the margo mucosae shifted more apically in all groups. Median changes ranged between -1.4 mm (-1.86; 0.21) for group T and -0.36 mm (-0.59; -0.17) for group Z1. Intragroup changes for groups without dehiscence defects were not statistically significant ($p>0.05$). The changes in groups Z1_D [median= -0.52 mm, (Q1= -0.65; Q3= -0.32)] and T_D [-1.26 mm, (-1.47; -0.5)], however, demonstrated a borderline statistical significance ($p=0.063$ for both groups). The group effect for the change of the level of MM was not statistically significant ($p>0.05$).

The median height of the peri-implant mucosa (MM-fBIC) in groups Z1_D [5.14 mm, (4.55; 5.23)] and T_D [5.43 mm, (5.19, 5.88)] was distinctively greater than in the other groups at 2 weeks. At 6 months of loading, the values of MM-fBIC were highest in groups Z1_D [4.51 mm, (4.3; 4.6)], followed by T_D [3.95 mm, (3.24; 4.31)], Z2, Z1 and T. Group Z1_D demonstrated statistically significantly differences compared to groups Z1, T and T_D ($p<0.05$). The change of MM-fBIC between 2 weeks and 6 months was statistically significant in group T_D [-1.45 mm, (-2.42; -1.06)] only. There was a statistically significant intergroup difference for the change of MM-fBIC between groups T_D and Z1 [0.58 mm, (0.19; 0.96), $p=0.006$] and between groups T_D and Z2 [0.42 mm, (-0.34; 1.13), $p=0.009$ (not-adjusted)].

The median length of the junctional epithelium (MM-aJE) was shorter for zirconia implants without dehiscence defect (0.88 and 0.62 mm for groups Z1 and Z2, respectively) than for all other groups at 2 weeks. At 6 months of loading, the median MM-aJE in groups Z1 (2.45 mm) and T (2.02 mm) were higher than in groups Z1_D (1.0 mm) and T_D (1.27 mm). Intergroup differences at 2 weeks and 6 months as well as changes between 2 weeks and 6 months were not statistically significant ($p>0.05$).

The corrected distances between IS and fBIC (IS-fBIC_c) in groups Z1_D (median= 2.81 mm, at 2-week healing, 2.65 mm at 6-month loading) and T_D (2.91 mm at 2-week healing, 2.01 mm at 6-month loading) were greater than in the other groups at both time points. The changes of IS-fBIC_c in all groups was not statistically significant over time ($p>0.05$), but there was a statistically significant intergroup difference between groups Z1 [1.04 mm, (0.84; 1.51)] and T_D [-0.71 mm, (-1.77; 0.68)] ($p=0.018$, not-adjusted).

***Bone-to-implant contact***

At 2 weeks of healing, the median percentage of the bone-to-implant contact (BIC) was highest in group Z1 [46.49, (37.15; 53.10)], followed by T_D, T, Z1_D and Z2, and there was statistically significant difference between groups Z1 and Z2 ($p$=0.0296, not-adjusted). At 6 months of loading, the median percentages of BIC in groups Z1 [83.53, (80.24; 88.38)] and Z1_D [85.39, (82.31; 90.08)] were higher than other groups (borderline $p$ value=0.058).

**Radiographic analysis**

In the 2-week healing groups, the median value of the change of the marginal bone level (MBchange) was minimal. In the 6-month loading groups, MBchanges between implant placement and crown insertion were statistically significant in groups Z1 [-0.8 mm, (-0.99; -0.62)] and T [-0.52 mm, (-0.82; -0.02)] ($p$=0.031 for both groups). However, MBchanges between crown insertion and sacrifice and between implant placement and sacrifice were not statistically significant in all groups ($p$>0.05). No statistically significant group effect was found ($p$>0.05) (Table 3).

## Discussion

The present study evaluating hard and soft tissue integration of two-piece titanium and zirconia dental implants with and without buccal dehiscence defects demonstrated that (i) the level of the margo mucosae shifts more apically over time in all group (but to an extent of borderline statistical significance in groups with dehiscence defects; Z1_D and T_D), (ii) the height of the peri-implant mucosa in groups Z1_D and T_D was greatest both at 2 weeks and 6 months, iii) the change in height of the peri-implant mucosa was significant for group T_D and included a change in both hard and soft tissues, whereas in group Z1_D no significant changes were observed, (iv) Z1 implants enhanced osseointegration compared to Z2 and T implants and, (v) radiographic marginal bone levels in all groups were stable during the entire experimental period.

Several preclinical studies previously investigated loaded zirconia implants (Akagawa, Ichikawa, Nikai, Tsuru, 1993; Delgado-Ruiz, et al., 2014; Janner, et al., 2018; Kohal, Weng, Bachle, Strub, 2004; Thoma, et al., 2015; Thoma, et al., 2016). All those studies assessed hard tissue healing, i.e. osseointegration and marginal bone level change, but only a limited number of studies evaluated the soft tissue response (Kohal, et al., 2004; Thoma, et al., 2015). Moreover, no study has been performed to analyze tissue integration at zirconia implants with dehiscence defects under loading condition.

In a pilot study (Lim, et al., 2018), the present chronic buccal dehiscence model was validated. In that study, the level of a buccal dehiscence defect appeared to be stable around implants up to 6 weeks of healing. Moreover, there was no substantial decrease in the dimension of peri-implant mucosa in Z1_D and T_D even though a slight apical shift of the margo mucosae was observed. However, the findings were limited to an early healing phase (2 and 6 weeks only), leaving some doubts on the potential stability of the peri-implant mucosa following prosthesis insertion and subsequent loading.

In the present study, all groups demonstrated an apical shift of the margo mucosae. Such changes (irrespective of the implant materials) might be due to tissue maturation, prosthetic procedures and loading. Even though, only groups Z1_D (median= -0.52, Q1= -0.65; Q3= -0.32) and T_D exhibited a borderline statistical significance, the median values of this apical shift were more pronounced around titanium (median= -1.4; Q1= -1.86; Q3= 0.21 mm for group T, median= -1.26; Q1= -1.47; Q3= -0.5 mm for group T_D) than for zirconia implants. Clinically, untreated implant dehiscence defects at titanium implants demonstrated to result in a stable margo mucosae on the short-term, but a higher risk to develop peri-implant disease on the long-run (Jung, et al., 2017; Schwarz, Sahm, Becker, 2012). The present preclinical study, provides first evidence that in case of untreated dehiscence defects, zirconia implants might be more favorable (less recession) than standard titanium implants if implant threads are left exposed. One can only speculate on the reasons for such differences: i) implant design, ii) implant surface, iii) soft tissue seal around the implant neck, iv) the number of leukocytes present around titanium and zirconia implants (Welander, et al., 2008), v) the number of bacteria present around titanium and zirconia implants (Rimondini, Cerroni, Carrassi, Torricelli, 2002).

Interestingly, observational studies demonstrated stable marginal gingival levels around titanium implants in case of missing buccal bone (Benic, et al., 2012; Kuchler, et al., 2016). Several reasons could be extrapolated for this observed partial or complete loss of buccal bone plate, but the finding could be interpreted as being that soft tissue compensated for missing hard tissue. In contrast to the present investigation with chronic ridge defects and late implant placement, only immediately placed implants (with simultaneous guided bone regeneration) were included in those two studies, thereby adding an additional confounding factor.

Even though an apical shift of the margo mucosa was observed in both groups with untreated dehiscence defects (Z1_D, T_D), a significant change in the height of the peri-implant mucosa was only noted in group T_D. Both hard and soft tissue changes can be responsible. In the pilot experiment for the present study, a minimal hard tissue change was noted, but the limitations were a small number of experimental animals, a short observation period and no loading of the implants (Lim, et al., 2018). In the present study, even though group T_D demonstrated a median bone gain over time (0.71 mm), the soft tissue loss (median loss = 1.26 mm) eventually resulted in a significant loss of the height of the peri-implant mucosa. Moreover, less changes in both hard (median gain = 0.09 mm) and soft tissue levels (median loss = 0.52 mm) were noted in group Z1_D. In the above-mentioned clinical study, a re-entry procedure was performed for all implants in order to connect the abutments (Jung, et al., 2017), revealing approximately 42 % of the implants with untreated dehiscence defect loosing buccal bone. This contradictory finding is attributable to the concept of implant placement; bone-driven implant placement for the present preclinical study and prosthetically driven placement for the above clinical study. Nonetheless, in both studies, titanium implants were negatively influenced by the presence of buccal dehiscence defect.

Systematic review based on preclinical studies demonstrated that zirconia implants led to similar bone-to-implant contact (BIC) to titanium implant in most of the included studies (Manzano, Herrero, Montero, 2014). On the basis of the data from groups without dehiscence defects, zirconia implant groups (groups Z1, Z2) demonstrated comparable BIC values to titanium implants (group T). It was also noticeable that group Z1 yielded higher BIC values compared to group Z2 at both time points (median value: 46.49 vs. 36.66%; $p<0.05$ at 2 weeks, 83.53 vs. 74.28%; $p>0.05$ at 6 months loading). A modified zirconia implant surface (Z1) led to a faster and an enhanced osseointegration compared to the non-modified surface (Z2).

The marginal bone level changes of zirconia implants and titanium implants in the present study demonstrated stability for both implant materials. The statistically significant changes for Z1 and T between implant placement and crown insertion appeared to be a physiological adaptation of the marginal bone (Abrahamsson, Berglundh, Moon, Lindhe, 1999; Ericsson, Nilner, Klinge, Glantz, 1996). There was no further significant loss of marginal bone after crown insertion in all groups, irrespective of the presence of a dehiscence defect.

The limitation of the present preclinical study is different behavioral and physiologic responses of an animal model compared to a clinical situation. Any translation of the present data into daily practice should be done with care. Even though zirconia implant demonstrated a potential to maintain the height of the peri-implant mucosa in the presence of a buccal bone dehiscence defect, this cannot be generalized and directly transferred to human patients. Rather, on the basis of these preclinical results, clinical studies should be performed to evaluate a potential clinical benefit of zirconia implants on the level of the soft tissues.

## Conclusion

The margo mucosae shifted apically to a greater extent at titanium than at zirconia implants with dehiscence defects. Despite concomitant hard tissue changes in both Z1 and T implants with dehiscence defects, a significant loss of the height of peri-implant mucosa was only observed for T implants. Osseointegration was enhanced in Z1 implant with a modified surface compared to Z2 implant. Radiographic marginal bone levels were stable irrespective of implant material and the presence of a buccal dehiscence defect.

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## Figure legend

**Figure 1.** Detailed schedule of surgical and prosthetic procedures, and sacrifices

**Figure 2.** Representative clinical photographs. (A) five implants with/without bony dehiscence defect were placed and healing abutments were connected. (B) clinical healing after 8 weeks of healing (mild gingival inflammation was observed around T implant). (C) prefabricated CAD/CAM crowns were inserted. (D) clinical healing after 6 months of loading.

**Figure 3.** Histomorphometric assessments. (A) Linear measurement. (B) BIC. BIC: bone-to-implant contact, MM: margo mucosa, IS: implant shoulder, aJE: apical extension of the junctional epithelium, BC: bone crest, fBIC: the first bone-to-implant contact.

**Figure 4.** Representative histologic views at 2 weeks of healing and at 6 months of loading (van Gieson staining).

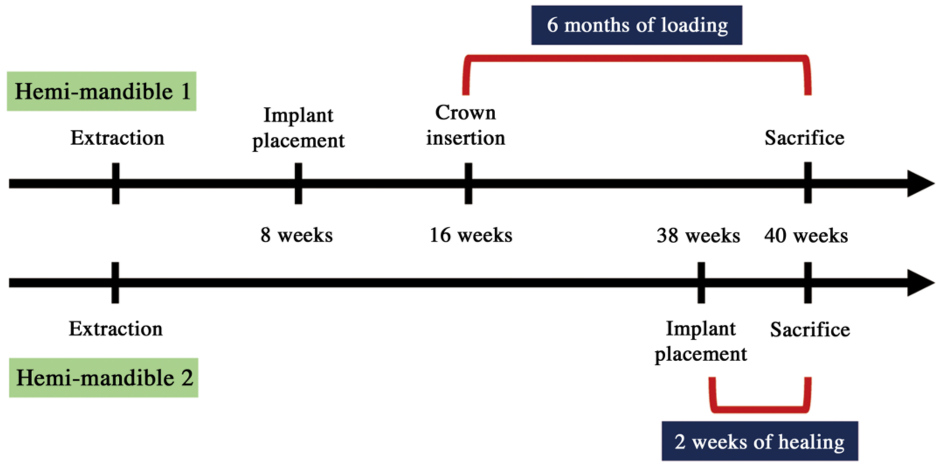


Figure 1

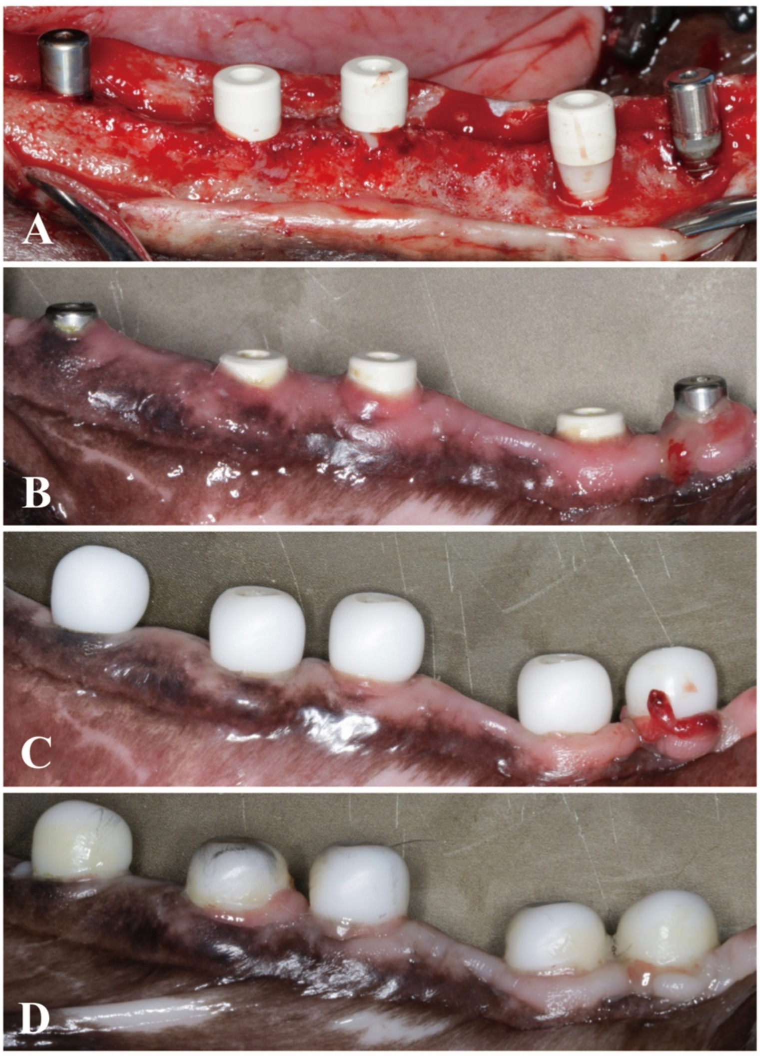


Figure 2

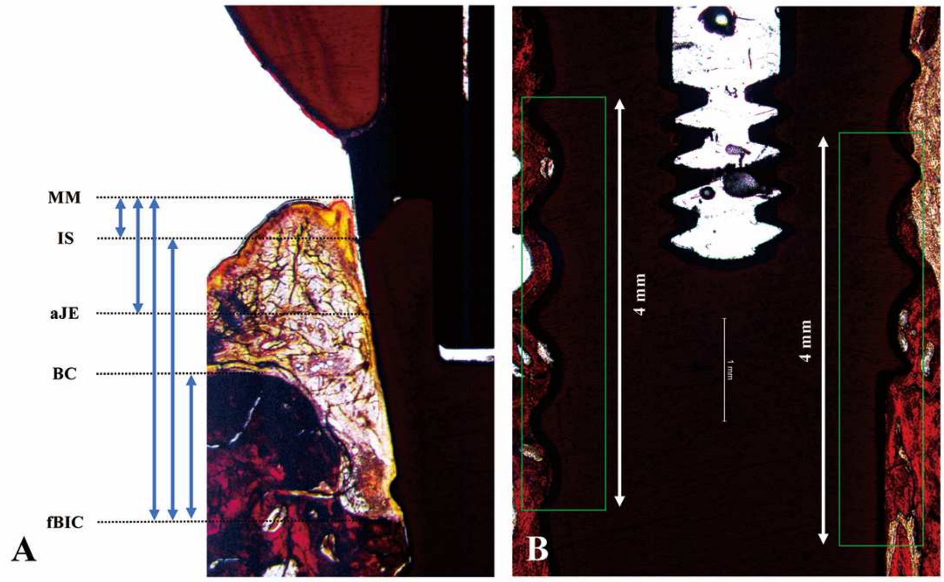


Figure 3

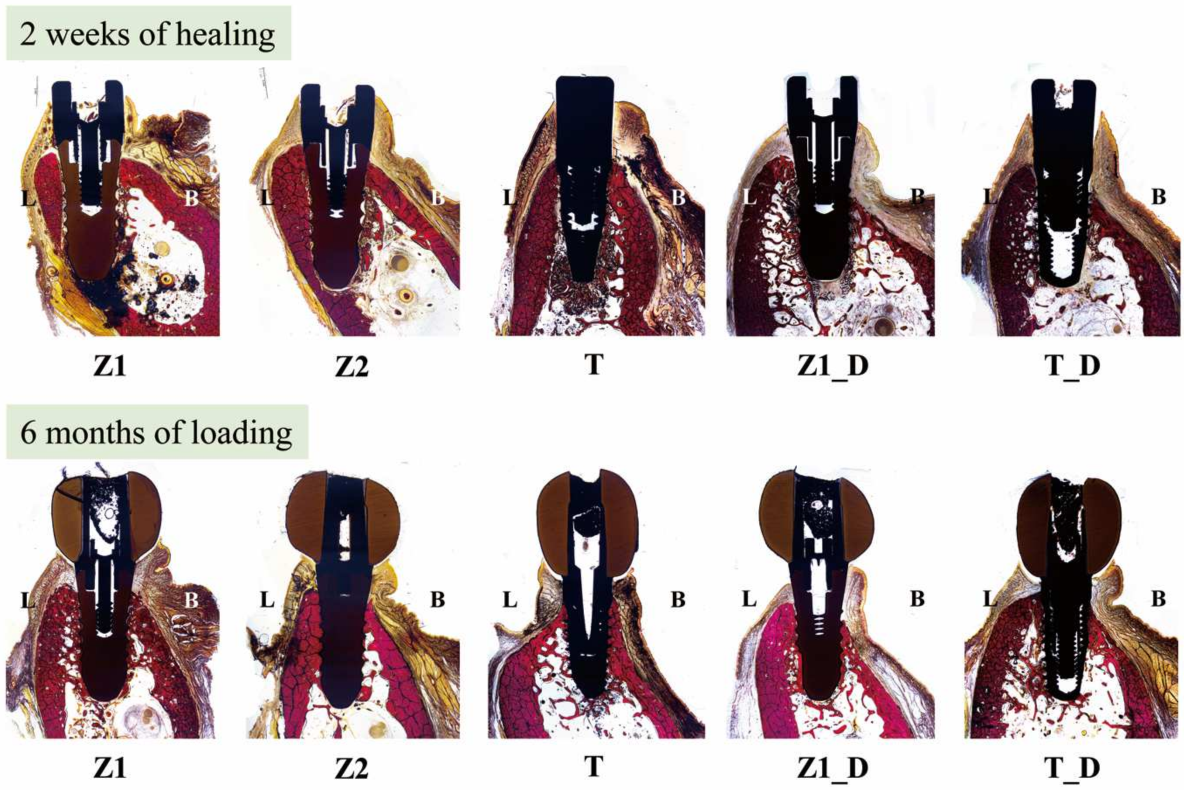


Figure 4

**Table 1.** Linear measurement on the soft tissue level

| | Z1 | Z2 | T | Z1_D | T_D |
|---|---|---|---|---|---|
| 2 weeks of healing [median (Q1; Q3), mean ± standard deviation] | | | | | |
| MM-IS | 1.85 (1.57; 2.07), 2.03±0.64 | 1.64 (1.44; 2.02), 1.97±0.93 | 2.64 (1.68; 3.47), 2.61±0.88 | 1.63 (1.57; 3.09), 2.09±0.78 | 2.15 (1.92; 2.44), 2.19 ±0.57 |
| MM-aJE | 0.88 (0.29; 1.01), 0.86±0.76 | 0.62 (0; 1.65), 0.79±0.85 | 1.63 (0.08; 2.04), 1.49±1.34 | 1.0 (0.56; 2.57), 1.45±1.28 | 1.90 (1.14; 2.65), 2.02±1.38 |
| MM-fBIC | 3.29 (2.88; 4.04), 3.52±0.85 | 3.29 (2.90; 3.78), 3.42±0.63 | 3.74 (3.08; 4.02), 3.80±2.85 | 5.14 (4.55; 5.23), 5.14±1.33 | 5.43 (5.19; 5.88), 5.44±0.75 |
| IS-fBIC_c* | 0.80 (0.43; 1.02), 0.78±0.48 | 0.64 (0.31; 1.31), 0.74±0.60 | 0.87 (0.77; 1.11), 0.78±0.51 | 2.81 (2.31; 2.96), 2.34±1.43 | 2.91 (2.53; 3.16), 2.85±0.31 |
| BC-fBIC | 0.21 (0.09; 0.97), 0.44±0.46 | 0.07 (0; 0.46), 0.34±0.55 | 0.2 (0.04; 0.51), 0.25±0.23 | 0.04 (0; 0.4), 0.15±0.21 | 0.21 (0.13; 0.44), 0.42±0.56 |
| 6 months of loading [median (Q1; Q3), mean ± standard deviation] | | | | | |
| MM-IS | 1.30 (1.10; 1.49), 1.46±0.87 | 1.49 (1.11; 1.99), 1.77±1.02 | 1.63 (1.13; 2.11), 1.76±0.85 | 1.23 (1.01; 1.58), 1.51±0.84¶ | 1.27 (0.21; 1.60), 1.12±0.80¶ |
| MM-aJE | 2.45 (1.0; 3.11), 2.09±1.26 | 0.85 (0.52; 1.37), 1.11±0.82 | 2.02 (0.89; 2.11), 1.86±0.88 | 1.0 (0.44; 1.98), 1.28±1.16 | 1.27 (0.29; 1.60), 1.37±1.21 |
| MM-fBIC* | 3.76 (3.61; 4.24), 3.88±0.46 | 3.86 (3.14; 4.80), 3.83±1.0 | 3.59 (3.27; 3.73), 3.33±0.67 | 4.51 (4.30; 4.60), 4.44±0.44 | 3.95 (3.24; 4.31), 3.83±0.58¶ |
| IS-fBIC_c* | 1.76 (1.54; 2.40), 1.72±0.73 | 0.82 (0.46; 2.33), 1.36±1.19 | 1.14 (0.49; 1.58), 1.17±0.87 | 2.65 (1.90; 2.83), 2.23±0.95 | 2.01 (1.23; 3.60), 2.30±1.26 |
| BC-fBIC | 0.09 (0; 0.42), 0.18±0.23 | 0.58 (0.40; 0.71), 0.72±0.61 | 0 (0; 0), 0.01±0.02¶ | 0.23 (0.12; 0.45), 0.34±0.36 | 0 (0; 0), 0±0 |
| Change between two time points | | | | | |
| MM-IS | -0.36 (-0.58; -0.17), -0.5±1.38 | -0.62 (-0.67; 0.67), -0.21±1.47 | -1.40 (-1.86; 0.21), -0.86±1.44 | -0.52 (-0.65; -0.32), -0.58±0.52 | -1.26 (-1.47; -0.50), -1.06±0.81 |
| MM-aJE | 1.90 (1.12; 3.01), 1.60±1.68 | 0.54 (-0.92; 1.37), 0.32±1.16 | 0.68 (-0.45; 1.97), 0.37±1.79 | -0.04 (-1.08; 1.23), -0.17±1.76 | -0.62 (-2.19; 1.27), 0.37±1.79 |
| MM-fBIC | 0.58 (0.19; 0.96), 0.47±1.04 | 0.42 (-0.34; 1.13), 0.41±1.15 | -0.35 (-1.77; 0.64), -0.47±1.18 | -0.54 (-1.53; -0.13), -0.69±1.59 | -1.45 (-2.42; -1.06), -0.47±1.18† |
| IS-fBIC_c | 1.04 (0.84; 1.51), 0.95±1.11 | 0.68 (-0.85; 1.80), 0.62±1.46 | 0.37 (-0.31; 0.94), 0.39±0.80 | -0.09 (-1.06; 0.52), -0.11±2.08 | -0.71 (-1.77; 0.68), -0.55±1.40 |
| BC-fBIC | -0.14 (-0.93; 0.29), -0.26±0.57 | 0.31 (0.17; 0.66), 0.39±0.88 | -0.20 (-0.49; -0.04), -0.24±0.22 | 0.09 (-0.08; 0.33), 0.19±0.45 | -0.21 (-0.44; -0.13), -0.42±0.56 |

Z1: zirconia implant with modified surface, placed within the bony housing, Z2: zirconia implant without modified surface, placed within the bony housing, T: titanium implant placed within the bony housing, Z1_D: Z1 implant placed with 3 mm of buccal bone dehiscence, T_D: titanium implant placed with 3 mm of buccal bone dehiscence, MM: the margo mucosa, IS: the implant shoulder, aJE: the apical extension of the junctional epithelium, fBIC: the first bone-to-implant contact, BC: the bone crest, IS-fBIC_c: the distance between IS and fBIC, subtracted by 0.7mm (zirconia implant) or 0.4mm (titanium implant).

[*]statistically significant group effect within each time point

[¶]statistically significant time effect in each group

[†]statistically significant change compared to “0” change

**Table 2.** Bone-to-implant contact ratio (%)

| Z1 | Z2 | T | Z1_D | T_D |
|---|---|---|---|---|
| 2 weeks of healing [median (Q1; Q3), mean ± standard deviation] * | | | | |
| 46.49 (37.15; 53.10), 46.9±12.14 | 36.66 (32.54; 40.52), 35.77±8.14 | 42.15 (35.94; 56.78), 46.05±11.78 | 36.90 (32.85; 47.28), 37.18±10.89 | 42.74 (36.75; 64.02), 47.43±14.66 |
| 6 months of loading [median (Q1; Q3), mean ± standard deviation] | | | | |
| 83.53 (80.24; 88.38), 81.48±14.26¶ | 74.28 (65.4; 90.79), 75.34±17.95¶ | 78.14 (63.45; 78.58), 74.65±10.76¶ | 85.39 (82.31; 90.08), 83.7±13.19¶ | 77.92 (66.14; 83.52), 74.97±11.98¶ |

Z1: zirconia implant with modified surface, placed within the bony housing, Z2: zirconia implant without modified surface, placed within the bony housing, T: titanium implant placed within the bony housing, Z1_D: Z1 implant placed with 3 mm of buccal bone dehiscence, T_D: titanium implant placed with 3 mm of buccal bone dehiscence, MM: the margo mucosa, IS: the implant shoulder, aJE: the apical extension of the junctional epithelium, fBIC: the first bone-to-implant contact, BC: the bone crest, IS-fBIC_c: the distance between IS and fBIC, subtracted by 0.7mm (zirconia implant) or 0.4mm (titanium implant).

*statistically significant group effect within each time point

¶statistically significant time effect in each group

**Table 3.** Changes of radiographical marginal bone level

| | Z1 | Z2 | T | Z1_D | T_D |
|---|---|---|---|---|---|
| 2 weeks of healing [median (Q1; Q3), mean ± standard deviation] | | | | | |
| Implant placement -Sacrifice | -0.07 (-0.47; 0.26), -0.33±0.94 | -0.30 (-0.35; -0.03), -0.24±0.22 | -0.17 (-0.43; -0.16), -0.24±0.21 | -0.15 (-0.36; -0.1), -0.28±0.46 | -0.14 (-0.4; 0.05), -0.1 ±0.35 |
| 6 months of loading [median (Q1; Q3), mean ± standard deviation] | | | | | |
| Implant placement –Crown insertion | -0.8 (-0.99; -0.62), -0.7±0.28† | -0.72 (-0.84; -0.32), -0.45±0.73 | -0.52 (-0.82; -0.02), -0.47±0.39† | -0.12 (-0.24; 0.04), -0.29±0.83 | -0.05 (-1.25; 0.18), -0.25±0.91 |
| Crown insertion -Sacrifice | 0.41 (-0.05; 0.49), 0.28±0.34 | 0.02 (-0.17; 0.15), 0.02±0.24 | 0.06 (-0.01; 0.31), 0.21±0.44 | -0.49 (-1.04; 0.15), -0.41±0.73 | 0.13 (-0.01; 0.87), 0.33±0.45 |
| Implant placement -Sacrifice | -0.49 (-0.67; -0.3), -0.5±0.54 | -0.51 (-1.02; -0.33), -0.44±0.68 | -0.14 (-0.67; -0.03), -0.26±0.41 | -0.91 (-1.44; 0.38), -0.7±0.9 | 0.18 (-1.31; 0.84), 0.07±1.29 |

Z1: zirconia implant with modified surface, placed within the bony housing, Z2: zirconia implant without modified surface, placed within the bony housing, T: titanium implant placed within the bony housing, Z1_D: Z1 implant placed with 3 mm of buccal bone dehiscence, T_D: titanium implant placed with 3 mm of buccal bone dehiscence, MM: the margo mucosa, IS: the implant shoulder, aJE: the apical extension of the junctional epithelium, fBIC: the first bone-to-implant contact, BC: the bone crest, IS-fBIC_c: the distance between IS and fBIC, subtracted by 0.7mm (zirconia implant) or 0.4mm (titanium implant).

†statistically significant change compared to “0” change